References:
Berends, B.R.; Urlings, H.A.P.; Snijders, J.M.A.; Van Knapen, F. (1996): Identification and quantification of risk factors in animal management and transport regarding *Salmonella* sp. in pigs. International Journal of Food Microbiology, 30. 37 – 53.

Castagna, S.M.F.; Cardoso, M.R.I. (2001): Association between *Salmonella* positive mesenteric lymph node/intestinal contents and contamination of tonsils/head lymph nodes of pigs at slaughter. Proc. 4th International Symposium On The Epidemiology And Control Of Salmonella In Pork. Leipzig, Germany. 310 - 312.

Funk, J.A.; Davies, P.R.; Nichols, M.A. (2001): Longitudinal study of *Salmonella enterica* in growing pigs reared in multiple-site swine production systems. Veterinary Microbiology, 83. 45 – 60.

Kich, J. D. (2003): Personal Comunication.

Kranker, S.; Alban, L.; Boes, J.; Dahl, J. (2002): Longitudinal investigation of *Salmonella* Typhimurium in integrated swine herds. Proc. 17th IPVS Congress. Iowa, USA. 317.

Michael, G.; Costa. M.; Cardoso. M. (1999): Comparison of selenite cystine broth, tetrathionate broth and Rappaprt Vassiliadis broth for the recovery of Salmonella from swine feces. Proc. 3th International Symposium On The Epidemiology And Control Of Salmonella In Pork. Washington, USA. 77 – 79.

Swanenburg, M.; Van Der Wolf, P.J.; Urlings, H.A.P.;Snijders, J.M.A.; Van Knapen, F. (2001): *Salmonella* in slaughter pigs: the effect of logistic slaughter procedures of pigs on the prevalence of *Salmonella* in pork. International Journal of Food Microbiology, 70. 231 – 242.

Van Der Gaag, M.A.; Vos, F.; Saatkamp, H.W.; Van Boven, M.; Van Beek, P.; Huirne, R.B.M. (2003): A state-transition simulation model for the spreadof Salmonella inthe pork supply chain. European Journal of Operational Research,. Article in Press.

Table 1. *Distribution of pigs according to bacteriological and serological results at the finishing phase (n=56) and at slaughter (n=26).*

	Finishing		Slaughter	
	Isolation Positive	Isolation Negative	Isolation Positive	Isolation Negative
ELISA Positive	13	3	3	17
ELISA Negative	29	11	2	4

PH 01 Prevalence and number of *Salmonella* in retail pork sausages

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Summary: The aim of this study was to assess the prevalence of *Salmonella* in Irish pork sausage at retail level. Samples, comprising branded prepacked sausages, loose sausages from supermarket meat counters and butcher shops, were collected from selected retail sites in four cities from October to December 2001 and from June to August 2002. A 3-tube Most Probable Number (MPN) method was used to enumerate *Salmonella* in a selected number of samples, which were positive by enrichment. *Salmonella* serotypes were detected in 4.4% and 1.7% of samples at each of the respective sampling periods; a level similar to those reported in other U.S. and U.K. studies. Limited results available on enumeration suggest that contamination rates were low. This study revealed that *Salmonella* are present in a proportion of Irish sausages and further risk analysis work is necessary in order to quantify

the risk posed to public health. Keywords: control programme, enumeration, serology, bacteriology, food safety.

Introduction: In Ireland, the crude incidence rate of reported human cases of salmonellosis, over 60% of which were due to *Salmonella enterica* serotype Typhimurium, rose in the 1990s to peak in 1998. Worldwide, the principal sources of *Salmonella* infection in humans are poultry, poultry products and eggs. However in Ireland control of *Salmonella* infection in the poultry industry has been highly effective as evidenced by the decreasing rate of isolation of *Salmonella* Typhimurium or *Salmonella* Enteritidis in chickens. Nevertheless, these serotypes represent the predominant serotypes associated with human infection in Ireland (FSAI, 2000). This gives rise to concern that there may be significant sources of *Salmonella* infection in Ireland other than poultry. A National *Salmonella* control programme in the pork industry was enacted in Ireland in August 2002.This study was undertaken as part of a larger project investigating the role of pork as a source of human salmonellosis in Ireland. The objectives of this study were 1) to determine the prevalence of *Salmonella* in pork sausage and 2) to ascertain contamination levels of *Salmonella* in sausage as part of the assessment of the role of pork as a source of human salmonellosis in Ireland.

Materials and Methods:
Retail Market survey Half-pound (200g) samples of fresh pork sausage were collected at weekly intervals over 8-week periods in 4 cites; Dublin, Cork, Galway and Athlone. Four hundred and fifty five samples were purchased at supermarkets (branded prepacked and loose-type) and butcher shops during the months of October to December 2001, while 466 samples were purchased during the months of June to August 2002. A total of eight supermarkets (different retail chains) and up to forty-eight butcher shops were sampled in each city. Samples were stored in a chilled container during transport and kept at 4AC prior to examination within twenty-four hours of purchase.
Microbiological analysis *Salmonella* isolation procedures were performed on 25g of each sausage sample according to BS EN 12824; 1998. Briefly, samples were pre-enriched in 225ml BPW and incubated for 16-24h at 37AC, followed by selective enrichment for 18h-24h in both Rappaport-Vassiliadis broth and selenite-cysteine broth at 41.5AC and 37AC respectively. Samples were plated onto mannitol lysine crystal violet brilliant green agar (MLCB) and brilliant green agar (BG) after both 24h and 48h of selective enrichment. Up to 5 suspect colonies per plate were identified by subculture onto MacConkey agar and inoculation of triple sugar iron agar slopes followed by serotyping.
MPN (Most Probable Number) Analysis An estimation of the number of *Salmonella* spp. in a selected number of samples was determined using a modified 3-tube MPN method (Dufrenne et al. 2001). These samples had been stored at –20AC for between 8 - 20 weeks.
Isolation of *Salmonella* serotypes from 3x50ml, 3x5ml and 3x0.5ml aliquots of homogenised sample in BPW was performed as described above. After confirmation, the number of *Salmonella* present in each sample was calculated using the MPN table of de Man (De Man 1983).

Statistical Analysis *Salmonella* prevalence was reported as the percentage of samples that tested positive. Differences in prevalence between product types, cities and time were compared using the chi square option of the frequency procedure of the Statistical Analysis Systems (SAS) and the Fischer's Exact Test.

Results: A summary of the *Salmonella* isolation rates is shown in Figure 1.During Part 1 of the survey (Oct-Dec 2001) 20/455 (4.4%) samples were positive for *Salmonella*. A total of 466 samples were tested in Part 2 of the study (June – Aug 2002) and 7 of these (1.5%) were positive for *Salmonella*. There was no significant difference in *Salmonella* prevalence between the four cities, from which samples were collected. However, there was a trend for butcher shop samples to be contaminated more often than the prepacked samples (P <0.07). Significant differences were observed between the two time points in the survey; a higher prevalence of *Salmonella* was reported in the winter months of the survey (P<0.02).

In total, 27 *Salmonella* isolates were identified. S. Typhimurium accounted for 23 of the isolates. S.Derby was isolated on two occasions while S. Livingstone and S. Bredeney were each isolated once.
Salmonella counts on twelve samples, which were positive for *Salmonella* when first examined, ranged from 1.5 to 37 organisms per 25g of sausage meat examined. In 50% of samples the pathogen was reduced to undetectable levels (< 0.3 MPN per 25g).

Discussion: The results of this study indicate that the rate of *Salmonella* contamination in Irish retail pork sausage samples is low (<5%). The detection rate was similar to other studies, which examined pork products, where *Salmonella* contamination was detected at levels ranging from 3.3% to 9.1% (Zhao et al., 2001, Mattick et al., 2002). The difference in prevalence levels could be due in part to the types of samples analysed i.e. comminuted product versus pork chop and also whether samples were fresh or frozen.
Outbreaks and clusters of human salmonellosis in Ireland show a marked seasonality with the highest number of cases occurring during the summer months (June to August). However, in this study the rate of contamination was significantly higher in winter months. No plausible explanation for this result can be offered, however similar findings were reported by Stock & Stolle, (2001) and Zhao et al., (2001).
Eighty five per cent of positive isolates recovered in this study were serotyped as Typhimurium. The predominance of S. Typhimurium is consistent with the results of other recent studies, which have examined pork and pork products (Stock et al., 2001). The presence of this serotype in pork is a cause of concern as S. Typhimurium is the top cause of human salmonellosis in Ireland.
Quantitative analysis of selected samples revealed that the number of *Salmonella* organisms in contaminated sausages was low (<1.5 to 40 per 25g). In this study pathogen levels were reduced to undetectable levels in 50% of samples. It is possible that initial counts of *Salmonella* in fresh samples were extremely low and therefore less likely to survive freezing. This survey confirms that a percentage of raw pork sausage meat in Ireland is contaminated with *Salmonella* and may lead to foodborne disease due to undercooking and/or cross contamination. The enactment into law of the National *Salmonella* control programme in Ireland (2002) should help to decrease the number of positive carcasses entering the abattoirs and thus reduce the prevalence of *Salmonella* in retail pork products.

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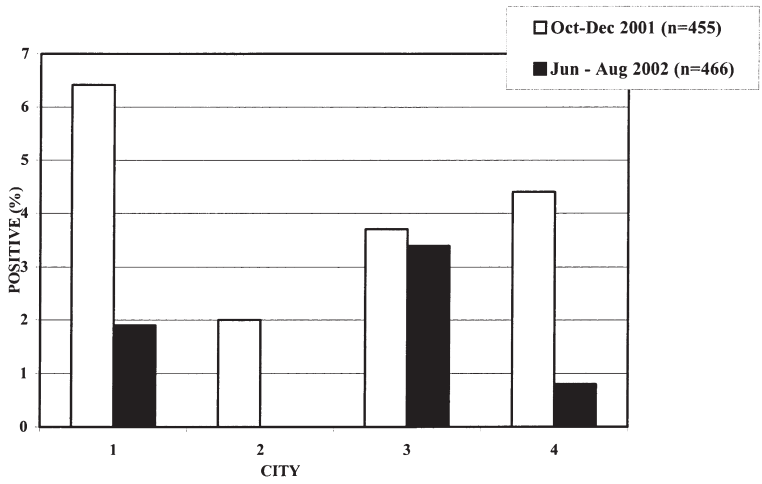
Figure 1. *Prevalence of Salmonella in samples of pork sausage collected in 4 cities on two occasions*



References:
Berends, B. R., Van Knapen, F., Mossel, D. A., Burt, S. A., Snijders, J. M. (1998): Salmonella spp. on pork at cutting plants and at the retail level and the influence of particular risk factors. Int J Food Microbiol 44, 207-17.

De Man, J. C. (1983): MPN tables, corrected. Europ. J. Appl. Microbiol. 17, 301-305.

Duffy, G.,Cloak, O.M.,O'Sullivan, M.G.,Guillet, A.,Sheridan, J. J.Blair, I. S.McDowell, D. A: (1999). Incidence and antibiotic resistance profiles of *Salmonella* spp. on Irish retail meat products. Food Microbiol. 16, 623-631.

Dufrenne, J.,Ritmeester, W.,Delfgou-van-Asch, E.,Van Leusden, F. M.De Jonge, R. (2001): Quantification of the Contamination of chicken and chicken products in the Netherlands with Salmonella and *Campylobacter*. J Food Prot 64, 538-541.

Epling, L.K.,Carpenter, J.A.,Blankenship, L.C. (1993): Prevalence of *Campylobacter* and *Salmonella* on pork carcasses and the reduction effected by spraying with lactic acid. J. Food Prot. 56, 536-537.

Hald, T. and H. C. Wegener (1999): Quantitative assessment of the sources of human salmonellosis attributable to pork. Proceedings of the 3rd International Symposium on Epidemiology and Control of *Salmonella* in Pork 4-7 August 1999, Washington, USA: 200-205.

Korsak, N.,Daube, G.,Ghafir, Y.,Chahed, A.,Jolley, S.,Vindevogel, H. (1998): An efficient sampling technique used to detect four food borne pathogens on pork and beef carcasses in nine Belgian abattoirs. J Food Prot 61, 535-541.

Lammerding, A.M.,Garcia, M.M.,Mann, E.D.,Robinson, Y.,Dorward, W.J.,Truscott, R.,Tittinger, F. (1988): Prevalence of salmonella and thermophyllic campylobacter in fresh pork. J Food Prot 51, 47-52.

Stock, K. and A. Stolle (2001): Incidence of *Salmonella* in minced meat produced in a European Union-approved cutting plant. J Food Prot 64, 1435-8.

Woods, R. L. and R. Rose (1992): Population of *Salmonella typhimurium* in internal organs of experimentally infected carrier swine. Am. J. Vet. Res. 53, 653-657.

Zhao, C.Ge, B.De Villena, J.Sudler, R.Yeh, E.Zhao, S.White, D. G.Wagner, D.Meng, J (2001): Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. Appl Environ Microbiol 67, 5431-6.

Comparison of an excision and a sponge sampling method for measuring salmonella contamination of pig carcasses

PD 01

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Summary: The aim of this study was to determine if an excision sampling method and a sponge sampling method give comparable results when trying to isolate salmonella from pig carcasses. During ten sampling days in one abattoir in total 312 carcasses were sampled; each carcass was sampled with both sampling methods to get paired observations. The number of salmonella positive excision samples (31 of 312) was significantly higher (P=0.00013) than the number of salmonella positive sponge samples (9 of 312). Sensitivity of the sponge method compared to the excision method was 6.5% and the comparability of both tests was low (kappa value was 0.08). As it seems that salmonella contamination levels of fresh pork are highly underestimated with the actually used sampling methods, the authors recommend that EU-authorities prescribe a destructive salmonella test for monitoring pig carcasses after slaughter in all EU-countries or a swab/sponge method with a comparable sensitivity.